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Losing Their Minds: Mena/VASP/EVL Triple Knockout Mice

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A recent *Neuron* paper by Kwiatkowski et al. that analyzes the phenotypes of mouse embryos lacking all members of the Ena/VASP family reveals new roles for these molecules in cortical development.

Ena/VASP proteins are a conserved family of actin-binding proteins implicated in a wide variety of processes that include intracellular movement of *Listeria*, phagocytosis, cell migration, and axon guidance (Krause et al., 2003). The full scope of Ena/VASP functions during mammalian development might have eluded investigators because the three vertebrate family members, Mena, VASP, and EVL, may provide overlapping functions that mask the effects of mutations in single or double mutants. In a recent issue of *Neuron*, Kwiatkowski et al. (2007) address this concern by generating and analyzing mice lacking all three family members. This study examines cortical development of these triple mutant mice.

Triply mutant animals (mmvvee mice) progressed to late embryogenesis prior to dying and displayed two primary cortical defects: a "cobblestone cortex" and the absence of major cortical axon tracts (Kwiatkowski et al., 2007). Cobblestone cortex results from breaks in the basal lamina of the pial membrane that encases the brain. These breaks allow cortical neurons to migrate out of the cortex and onto the surface of the brain, causing stereotypical bumps known as ectopias. Similar neuronal ectopias were seen in conditional mouse knockouts of focal adhesion kinase (FAK) (Beggs et al., 2003). Results from the FAK analysis were consistent with previous studies concerning the role of radial glial cells, which extend across the cortical layers, in basal lamina integrity (Beggs et al., 2003). Kwiatkowski et al. (2007) found that radial glial endfeet were not tightly associated with the pial membrane in mmvvee mice, and based on this phenotype and previous studies, they proposed that the neuronal ectopias in mmvvee mice resulted from defects in glial endfoot adhesion. These ectopias may arise from defects in adhesion between glia and the pial membrane, or between neighboring glial endfeet at the pial membrane. Their data are consistent with a requirement for Ena/VASP in radial glia themselves, but a glia-specific knockout of Ena/VASP proteins will be required to test this hypothesis directly.

One surprise was that mmvvee mice had largely normal cortical layers in regions not affected by ectopias. The mouse cortex is organized into distinct layers that are populated by cortical neurons according to their age, with younger neurons undergoing longer migrations to more superficial layers. Previous work suggested that Ena/VASP proteins play a critical role in cortical neuron migration and hence proper positioning of neurons into their appropriate cortical layer (Goh et al., 2002). As previously observed, some neurons did migrate too far in mmvvee mice, but in contrast to the previous study, these mmvvee neurons remained in their proper layer (Kwiatkowski et al., 2007). Different experimental protocols may explain the observed differences between the two studies since the general phenotype in both was overmigrated neurons. Neuronal cell body and growth cone migrations in a variety of organisms rely on many of the same molecules, ranging from guidance cues and receptors to the mediators of actin-based motility. These relatively normal cortical cell migrations and the dramatic lack of cortical axons discussed below suggest that the ability of cortical neurons to migrate and to later extend a growth cone can be mechanistically distinct processes.

The most striking cortical defect seen in mmvvee mice was the near-complete absence of cortical axon tracts. Using mmvvee chimeric mouse embryos where the triply mutant cells were marked with GFP, the authors found that only the GFP+ mmvvee neurons failed to form axons in vivo, indicating that the missing axon tracts resulted from a cell-autonomous defect in axon formation. To determine the nature of this defect in axon development, the authors analyzed the development of the mutant neurons in culture. Axon formation in cultured cortical neurons can be divided into three stages. In stage 1, actin-rich filopodia and lamellipodia protrude in all directions from the neuronal cell body. In stage 2, these protrusions become organized into neurites, cell-body extensions with growth cones at their tips. At this stage, the neurite destined to become the axon cannot be identified. During stage 3, one neurite becomes the axon. All previously known mutations and drug treatments that disrupt neuritogenesis affect stages 2 and 3, precluding an analysis of the earliest stage (da Silva and Dotti, 2002). However, in this study most of the mmvvee neurons remained in stage 1. The authors discovered that the mutant cells often failed to form filopodia, f-actin bundles that are thought to provide force against the membrane, which promote protrusive outgrowth. Ena/VASP proteins are normally localized to the leading edges of migrating cells and to the tips of filopodia in fibroblasts and growth cones in migrating axons (Krause et al.,

2003). Though Ena/VASP molecules are best known for their ability to protect the barbed ends of f-actin from capping, they also appear to cluster the tips of actin filaments within filopodia (Applewhite et al., 2007), and this latter role is also likely to be important for filopodia formation and therefore neurite initiation.

Based on these observations, Kwiatkowski et al. (2007) propose tha filopodia are essential for neurite formation, a model that places Ena/VASP proteins as key regulators of this process. The *Drosophila* and *C. elegans* genomes each encode a single Ena/VASP family member, and while loss of these molecules leads to guidance defects, neurons still produce an axon (Krause et al., 2003). Kwiatkowski et al. (2007) state that outside the cortex, axon tracts still form in mmvvee mutants, which is consistent with the studies of invertebrate neurons. Whether these differences represent a fundamental difference between neuritogenesis in the cortex and in other parts of the nervous system will require further study.

The vast majority of mmvvee mice exhibited exencephaly, wherein the cerebral cortex is extruded outside of the brain cavity. While this exencephaly was not studied extensively, it likely results from defects in neural tube closure. This phenotype is not surprising for two reasons. First, mice lacking both copies of Mena and VASP exhibit exencephaly, demonstrating a role for Ena/VASP in neural tube closure (Menzies et al., 2004). Second, epithelial cells of *Drosophila* and *C. elegans* enclose developing embryos in a process that is analogous to neural tube closure. *Drosophila* Ena protein is found at the leading edge of migrating epithelial cells that mediate dorsal closure, and embryos deficient for *ena* have defects in the migration and matching of cells at the dorsal midline (Gates et al., 2007). The *C. elegans* Ena homolog, UNC-34, also localizes to the leading edge of migrating epithelial cells and, together with the Wiscott-Aldrich Syndrome homolog WSP-1, promotes ventral enclosure (Sheffield et al., 2007; Withee et al., 2004). As in cultured cortical neurons, the Ena and UNC-34 proteins promote filopodial projections at the leading edge of the migrating epithelial cells in flies and worms (Gates et al., 2007; Sheffield et al., 2007). Thus, the complete elimination of Ena/VASP family members in mouse, flies, and worms points to a pivotal role for this family of proteins in mediating the movements of epithelial cells during morphogenesis and suggests that these disparate developmental events might share mechanistic similarities.

Overall, the work of Kwiatkowski et al. (2007) contributes to our understanding of the diverse roles that Ena/VASP proteins play in the developing nervous system. Ena/VASP proteins are likely to be required in at least three cell types: in neuroepithelial cells for neural tube closure, in cortical neurons for neuritogenesis, and in radial glial cells for an intact pial membrane. Precisely how Ena/VASP proteins promote filopodial formation in cortical neurons—and why they are so critical to the regulation of axon, but not neuronal cell body, migration—are interesting questions that await further investigation. The mmvvee mice provide a new tool to address these issues.

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